SmartBioSurface® Coating

Advanced Optical Microscopy for Non-Adhering Cells

The Challenge: Imaging Non-Adherent Cells with Advanced Optical Microscopy

Advanced Optical Microscopy (AOM) has become an essential tool for studying cellular morphology, cytoskeletal organization, and biomarker localization. However, imaging non-adherent cells, such as hematological cell lines and primary white blood cells (WBCs), remains challenging because these cells lack intrinsic substrate attachment and are often lost during fixation, staining, and washing procedures [1].
Among advanced fluorescence microscopy techniques, including Confocal Laser Scanning Microscopy (CLSM) and spinning-disk confocal microscopy, stable cell immobilization, low background fluorescence, and high optical quality are essential for accurate visualization of cellular structures [1–3]. These requirements are even more critical for super-resolution microscopy techniques, such as Structured Illumination Microscopy (SIM) where image reconstruction relies on multiple illumination patterns and is highly sensitive to sample drift, motion, and optical background [2,3].
To improve the retention of suspension cells, adhesion-promoting coatings such as Poly-D-Lysine (PDL) are widely used. However, their effectiveness depends on cell type and experimental conditions, often limiting their performance in demanding imaging applications.
Therefore, the development of surface coatings that combine efficient immobilization of non-adherent cells with low autofluorescence and compatibility with advanced optical microscopy remains an important challenge for research applications.

The Solution: SmartBioSurface® Slides

SmartBioSurface is a proprietary **nanostructured titanium-based coating** [4] developed to promote **spontaneous adhesion** of non-adherent cells onto glass slides while **preserving cellular morphology** and structural integrity [5].

Figure1. SmartBioSurface slides. Left Image: SmartBioSurface slides in a different format. Right Image: Atomic Force Microscopy (AFM) of the nanocoating (50 nm thickness)

The coating consists of a nanostructured titanium dioxide (TiO_2) layer deposited on optical-grade glass. The combination of efficient cell immobilization and favourable optical properties makes slides a promising **tool**

for fluorescence microscopy workflows requiring extensive sample processing and **high-performance image** acquisition.

However, because the coating is composed of nanostructured TiO_2, its compatibility with advanced laser-based fluorescence microscopy techniques could not be assumed. Nanostructured surfaces may alter light propagation or generate substrate-related optical background, potentially affecting image quality and super-resolution image reconstruction. Therefore, the performance of SmartBioSurface slides was tested across advanced fluorescence microscopy modalities to determine whether the coating remains compatible with high-intensity laser illumination and demanding imaging conditions.

1. Materials & Methods

Cell Models

Human suspension cell lines Jurkat Clone E6-1 (ATCC® TIB-152™) and U937 (ATCC® CRL-1593.2™) were used as representative lymphoid and myeloid cell models, respectively. Primary white blood cells (WBCs) isolated from peripheral blood samples collected from healthy donors were included as a physiological reference population.

Imaging Platforms and SmartBioSurface Coated Supports

The compatibility of SmartBioSurface slides with advanced fluorescence imaging workflows was evaluated using confocal and super-resolution microscopy platforms. CLSM was performed on a Leica SP8 confocal microscope (Leica DMi8 inverted platform) equipped with a HC PL APO 63×/1.40 Oil CS2 objective. Spinning disk confocal microscopy and SIM were performed on a Nikon Eclipse Ti2 inverted microscope equipped with a CrestOptics X-Light V3 spinning disk confocal module and DeepSIM SIM system, using a CFI Plan Apochromat Lambda 60×/1.42 Oil objective.

SmartBioSurface slides (50 nm TiO_2 coating) were compared with Poly-D-Lysine (PDL)-coated glass slides as benchmark controls.

CLSM, spinning disk confocal microscopy, and SIM experiments enable confocal and super-resolution fluorescence imaging, including optical sectioning, three-dimensional reconstruction, and high-resolution visualization of subcellular structures. For confocal imaging, z-stack datasets were acquired and analyzed as individual optical sections and maximum intensity projections. SIM images were reconstructed using the instrument's standard super-resolution reconstruction workflow.

PBMC Isolation from a Peripheral Blood Sample

Peripheral blood samples collected from healthy donors [6] in EDTA tubes are processed to isolate leukocytes via red blood cell lysis.

Leukocyte count is determined using an automated hematology analyzer (HemoCue).

Whole blood is incubated with Red Blood Cell Lysis Buffer (RBL) for approximately 5 minutes at room temperature, allowing selective lysis of erythrocytes while preserving leukocytes. Following incubation, the sample is centrifuged at 249 g for 5 minutes to pellet the leukocyte fraction. The supernatant containing lysed erythrocytes is carefully discarded.

To ensure complete removal of red blood cells, the pellet is subjected to two additional lysis cycles. After the final centrifugation step, the leukocyte pellet is resuspended in Dulbecco's Phosphate Buffered Saline 1X without Ca and Mg (DPBS) (EuroClone, Milan, Italy) to achieve the proper cell density of up to approximately 250.000 cells/cm^2.

Cultured Suspension Cells: Jurkat; U937

Jurkat, Clone E6-1 (ATCC® TIB-152™) - human T lymphoblast-like suspension cell line derived from acute T-cell leukemia - and U937 (ATCC® CRL-1593.2™) - human pro-monocytic suspension cell line derived from histiocytic lymphoma - are collected directly from the culture flask by gentle pipetting and transferred into a centrifuge tube. The suspension is centrifuged at approximately 249g for 5 minutes, after which the supernatant is removed, and the cell pellet is resuspended in DPBS 1X (EuroClone, Milan, Italy).

Cell concentration is determined using a Bürker hemocytometer combined with a viability dye.

The cell suspension was adjusted to achieve a final seeding density of up to approximately 250,000 cells/cm^2 on the SmartBioSurface slides.

Cell Seeding on SmartBioSurface slides

Cell suspensions prepared as described above were dispensed directly onto SmartBioSurface slides. Samples were left undisturbed for approximately 20 minutes at room temperature, allowing cells to settle and spontaneously adhere to the nanostructured titanium surface.

This procedure promotes efficient immobilization of non-adherent cells while preserving cellular morphology and structural integrity. The stable attachment achieved on the titanium-coated surface supports subsequent sample processing steps and advanced fluorescence imaging applications.

Fixation

Following cell seeding and adhesion, samples were fixed with paraformaldehyde (PFA) at 4% for 20 minutes at room temperature.

After fixation, samples were gently rinsed with 1× DPBS and allowed to air dry completely. Fixed samples were then processed according to the subsequent staining and imaging workflows (Figure 2).

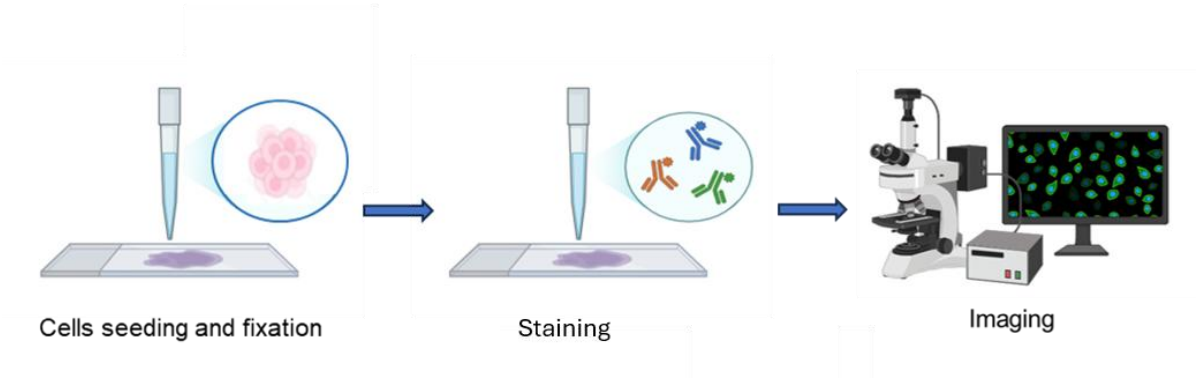

Figure 2. Schematic overview of the experimental workflow for fluorescence imaging, including cell seeding on SmartBioSurface slides, immunofluorescence staining, and image acquisition.

Immunofluorescence Staining

Slides were first allowed to equilibrate at room temperature and rehydrated by two consecutive incubations in DPBS 1X (EuroClone, Milan, Italy) for 5 min each in a humid chamber. Cells were then permeabilized with 0.1% Tergitol™ in DPBS 1X for 10 min at room temperature, followed by three washes in DPBS 1X (EuroClone, Milan, Italy) for 1 min each.

To minimize non-specific binding, samples were incubated with a blocking solution consisting of 5% Normal Goat Serum (NGS; Cell Signaling Technology) in DPBS 1X (EuroClone, Milan, Italy) for 1 h at room temperature.

For CLSM, Spinning Disk Confocal Microscopy, and SIM, directly conjugated antibodies against CD45 (BioLegend 393406) and CD66-Alexa Fluor® 647 (BioLegend 392912) were applied with Phalloidin-iFluor™ 488 (Abcam ab176753) and/or Phalloidin-iFluor™ 555 (Abcam ab176756) in blocking solution and incubated for 2 h at 37°C in a humid chamber.

Following staining, samples were washed three times with DPBS 1X (EuroClone, Milan, Italy) for 1 min each at room temperature. Nuclei were counterstained with DAPI for 10 min at room temperature, followed by a 5 min wash in DPBS 1X and a brief rinse in Milli-Q water. DAPI-only samples were prepared separately as negative controls for background fluorescence assessment.

Glass slide-based samples were air-dried under a biological hood for at least 15 min, protected from light, and mounted using ProLong™ Antifade Mountant (Thermo Fisher Scientific) with a glass coverslip. Mounted slides were allowed to cure at room temperature before image acquisition.

2. Results

Compatibility with Confocal and Spinning Disk Microscopy

Cells remained stably immobilized throughout fixation, permeabilization, immunofluorescence staining, and washing procedures on titanium-coated slides, enabling reproducible multicolour fluorescence imaging of Jurkat cells, U937 cells, and primary WBCs. Robust CD45/CD66 membrane staining, phalloidin-labelled actin, and DAPI nuclear staining were consistently detected without evidence of coating-related interference.

SmartBioSurface slides demonstrated excellent compatibility with confocal fluorescence microscopy. CLSM and Spinning Disk Confocal Microscopy enabled clear visualization of cellular morphology and fluorescent markers in Jurkat cells, U937 cells, and primary WBCs.

Representative images acquired on SmartBioSurface slides showed clear detection of CD45/CD66 membrane staining, phalloidin-labelled actin structures, and DAPI-labelled nuclei. Single optical sections and maximum-intensity projections provided consistent image quality across all tested substrates, allowing visualization of individual cells and subcellular features.

Comparable image quality, signal-to-noise ratio, and background fluorescence were obtained on titanium-coated and PDL-coated control slides, with no detectable coating-related imaging artifacts.

Across both confocal modalities, fluorescence intensity varied slightly between biological replicates but showed no consistent dependence on substrate condition. No substrate-dependent differences in background fluorescence or imaging artifacts were observed.

Spinning Disk Confocal Microscopy further confirmed the suitability of titanium-coated slides for rapid fluorescence imaging. Strong signal intensity and good image contrast were maintained across all fluorescence channels, enabling efficient visualization of membrane markers, actin cytoskeleton organization, and nuclear staining. Background fluorescence remained consistently low across all tested substrates, confirming the suitability of SmartBioSurface for rapid multicolor confocal imaging.

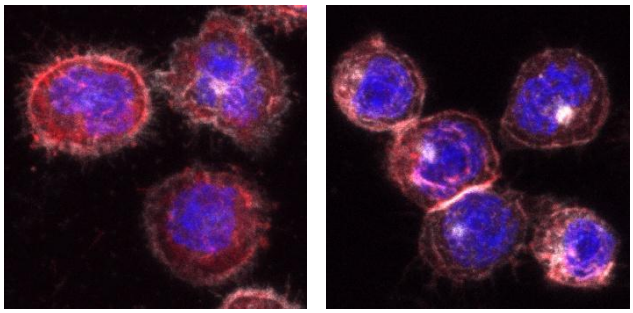

Figure 3. Confocal laser scanning microscopy maximum intensity projections of Jurkat cells prepared under different conditions (Poly-D-Lysine and SmartBioSurface from left to right). Cells were stained with CD45/CD66 (Alexa Fluor 647, white), Phalloidin (Alexa Fluor 555, red), and DAPI (blue). Images were acquired using a Leica SP8 confocal microscope (Leica DMi8 inverted platform) equipped with a HC PL APO 63×/1.40 Oil CS2 objective (2048 × 2048 pixels; z-stack acquisition with 0.3 µm z-step) using identical imaging parameters and are shown as maximum intensity projections.

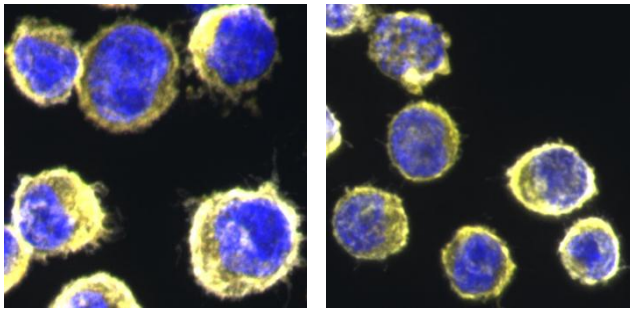

Figure 4. Spinning disk confocal microscopy maximum intensity projections of U937 cells prepared under different conditions (Poly-D-Lysine and SmartBioSurface from left to right). Cells were stained with CD45/CD66 (Alexa Fluor 647, white), Phalloidin (Alexa Fluor 555,

red), Phalloidin (Alexa Fluor 488, green), and DAPI (blue). Images were acquired using a Nikon Eclipse Ti2 inverted microscope equipped with a CrestOptics X-Light V3 spinning disk confocal module and a CFI Plan Apochromat Lambda 60×/1.42 Oil objective (2394 × 2394 pixels; z-stack acquisition with a 0.3 µm z-step) using identical imaging parameters and are shown as maximum intensity projections.

Super-Resolution Imaging by Structured Illumination Microscopy (SIM)

SIM imaging was successfully performed on SmartBioSurface slides. Reconstruction of super-resolved images enabled enhanced visualization of actin structures at the cell–substrate interface compared with conventional diffraction-limited imaging.

This improvement was particularly evident for fine cortical actin structures, cell protrusions, and adhesion-associated features. Fine cytoskeletal details were clearly resolved in Jurkat cells, U937 cells, and primary WBCs, demonstrating reliable performance during SIM acquisition and image reconstruction.

Image quality and reconstruction performance were comparable across standard titanium coatings, thin titanium coatings, and PDL-coated control substrates.

Importantly, no coating-dependent reconstruction artifacts or optical distortions were observed, indicating that the nanostructured titanium coating does not interfere with SIM image reconstruction and is fully compatible with super-resolution fluorescence microscopy.

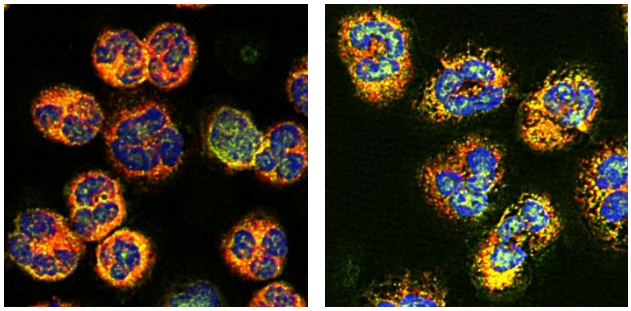

Figure 5. Structured illumination microscopy (SIM) images showing a zoomed view of the adhesion plane of white blood cells (WBCs) prepared under different conditions (Poly-D-Lysine and SmartBioSurface from left to right). Cells were stained with CD45/CD66 (Alexa Fluor 647, red), Phalloidin (Alexa Fluor 555, yellow), Phalloidin (Alexa Fluor 488, green), and DAPI (blue). Images were acquired using a Nikon Eclipse Ti2 inverted microscope equipped with the CrestOptics DeepSIM structured illumination microscopy system and a CFI Plan Apochromat Lambda 60×/1.42 Oil objective (1024 × 1024 pixels; single-plane acquisition) using identical SIM imaging parameters and are shown as zoomed views of the cell adhesion plane.

3. Conclusion

SmartBioSurface slides extend the benefits of nanostructured titanium-based cell immobilization to advanced fluorescence microscopy applications.

The coating provides:

- **Efficient adhesion** of non-adherent cells on imaging-grade slides.
- **Compatibility** with multicolor **immunofluorescence** staining workflows.
- Consistently **low background** fluorescence without detectable coating-derived fluorescence artifacts.
- **High-quality imaging** across advanced fluorescence microscopy platforms, including **CLSM** and **Spinning Disk** microscopy.
- **Compatibility** with **super-resolution** Structured Illumination Microscopy (SIM), enabling **high-quality image reconstruction** without coating-dependent optical artifacts.

Across all tested microscopy modalities, SmartBioSurface slides were fully compatible with multicolor fluorescence imaging and advanced laser-based microscopy workflows.

Overall, these results demonstrate that SmartBioSurface slides provide a versatile platform for fluorescence imaging of suspension-derived cells, combining efficient cell immobilization, cell retention, low background fluorescence, and excellent compatibility with confocal and SIM imaging. These features support high-quality fluorescence analysis across a wide range of advanced microscopy workflows.

References

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6. Clinical trial NCT05942066

SmartBioSurface®

Enabling Advanced Fluorescence Microscopy

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